

AGILENT TECHNOLOGIES, INC.  
Legal Department, DL429  
Intellectual Property Administration  
P. O. Box 7599  
Loveland, Colorado 80537-0599

ATTORNEY DOCKET NO. 10991398-1

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JUL 11 2007

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Diane D. Ilsley

Serial No.: 09/919,643

Examiner: Sue Xu Liu

Filing Date: July 31, 2001

Group Art Unit: 1639

Title: METHODS FOR DEPOSITING SMALL VOLUMES OF PROTEIN FLUIDS ONTO THE SURFACE  
OF A SUBSTRATE

COMMISSIONER FOR PATENTS  
P.O. Box 1450  
Alexandria VA 22313-1450

## TRANSMITTAL LETTER FOR RESPONSE/AMENDMENT

Sir:

Transmitted herewith is/are the following in the above-identified application:

- ☐ Response/Amendment ☐ Petition to extend time to respond  
☐ New fee as calculated below ☐ Supplemental Declaration  
☒ No additional fee (Address envelope to "Mail Stop Amendments")  
☒ Other: partially signed Declaration Under 37 C.F.R. 1.131 and Exhibit A (Fee \$ \_\_\_\_\_)

CLAIMS AS AMENDED BY OTHER THAN A SMALL ENTITY						
(1) FOR	(2) CLAIMS REMAINING AFTER AMENDMENT	(3) NUMBER EXTRA	(4) HIGHEST NUMBER PREVIOUSLY PAID FOR	(5) PRESENT EXTRA	(6) RATE	(7) ADDITIONAL FEES
TOTAL CLAIMS		MINUS		= 0	X 50	\$ 0
INDEP. CLAIMS		MINUS		= 0	X 200	\$ 0
<input type="checkbox"/> FIRST PRESENTATION OF A MULTIPLE DEPENDENT CLAIM					+ 380	\$ 0
EXTENSION FEE	1 <sup>st</sup> MONTH 120.00 <input type="checkbox"/>	2 <sup>nd</sup> MONTH 450.00 <input type="checkbox"/>	3 <sup>rd</sup> MONTH 1020.00 <input type="checkbox"/>	4 <sup>th</sup> MONTH 1590.00 <input type="checkbox"/>		\$ 0
OTHER FEES						\$ 0
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT						\$ 0

Charge \$ 0 to Deposit Account 50-1078. At any time during the pendency of this application, please charge any fees required or credit any over payment to Deposit Account 50-1078 pursuant to 37 CFR 1.25. Additionally please charge any fees to Deposit Account 50-1078 under 37 CFR 1.16, 1.17, 1.19, 1.20 and 1.21. A duplicate copy of this transmittal letter is enclosed.

Respectfully submitted,

Diane D. Ilsley

By

Bret E. Field for Ping Hwung  
Attorney/Agent for Applicant(s)

I hereby certify that this paper is being facsimile  
transmitted to the Patent and Trademark Office on  
the date shown below:

571-273-8300

Date of facsimile: July 11, 2007

Typed Name: Sandra D. Hunter

Signature: 

Reg. No. 37,620

Date: July 11, 2007

Telephone No. (650) 327-3400

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(1) FOR	(2) CLAIMS REMAINING AFTER AMENDMENT	(3) NUMBER EXTRA	(4) HIGHEST NUMBER PREVIOUSLY PAID FOR	(5) PRESENT EXTRA	(6) RATE	(7) ADDITIONAL FEES
TOTAL CLAIMS		MINUS		= 0	X 50	\$ 0
INDEP. CLAIMS		MINUS		= 0	X 200	\$ 0
<input type="checkbox"/> FIRST PRESENTATION OF A MULTIPLE DEPENDENT CLAIM					+ 360	\$ 0
EXTENSION FEE	1 <sup>ST</sup> MONTH 120.00 <input type="checkbox"/>	2 <sup>ND</sup> MONTH 450.00 <input type="checkbox"/>	3 <sup>RD</sup> MONTH 1020.00 <input type="checkbox"/>	4 <sup>TH</sup> MONTH 1590.00 <input type="checkbox"/>		\$ 0
OTHER FEES						\$ 0
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT						\$ 0

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Diane D. Ilsley

By

Bret E. Field for Ping Hwung  
Attorney/Agent for Applicant(s)

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<b>DECLARATION UNDER 37 C.F.R. §1.131</b>  Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Application Number	09/919,643
	Attorney Docket Number	10991398-1
	Filing Date	July 31, 2001
	First Named Inventor	Diane D. Ilsley
	Examiner	Sue Xu Liu
	Group Art	1639
	Title	Methods for Depositing Small Volumes of Protein Fluids Onto the Surface of a Substrate

This Declaration and the attached Exhibit are being submitted in conjunction with the Applicants' Response to the Office Action dated February 27, 2007.

We, Diane Ilsley, Douglas Amorese, Michael Caren and Peter Tsang, do hereby declare as follows.

1. We are the inventors of the invention claimed in the above captioned application.
2. We have been asked to declare and provide factual evidence in support of conception of the above captioned application before April 24, 2001.
3. As evidenced by Exhibit A, we conceived of using an inkjet printhead to deposit protein reagents, e.g., antibodies, prior to April 24, 2001. The dates have been redacted from Exhibit A. However, a redacted date set forth in Exhibit A shows conception of the invention prior to April 24, 2001.
4. Exhibit A consists of a photocopy of an Invention Disclosure (total of 9 pages) in which details of methods for depositing a quantity of fluid containing a protein of interest, e.g., an antibody, onto the surface of a substrate are described.

5. Pages 1 to 2 of Exhibit A are internal Invention Disclosure forms used by the Legal Department of Agilent Technologies. Pages 3 to 9 of Exhibit A, describe the details of the methods of the invention. In brief, methods for depositing a quantity of fluid containing a protein of interest, e.g., an antibody, onto the surface of a substrate is set forth. This invention allows for the deposition of a protein onto the surface of a substrate without substantially modulating the activity or functionality of the protein.
6. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: 7-11-07

Diane Hsley  
Diane Hsley

Date: \_\_\_\_\_

\_\_\_\_\_  
Douglas Amorese

Date: \_\_\_\_\_

\_\_\_\_\_  
Michael Caren

Date: \_\_\_\_\_

\_\_\_\_\_  
Peter Tsang

Attachments: Exhibit A

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Agilent Technologies

## INVENTION DISCLOSURE

PAGE ONE OF

PONO 10003540 DATE RCVD

ATTORNEY GMS/BSP

Instructions: The information contained in this document is COMPANY CONFIDENTIAL and may not be disclosed to others without prior authorization. Submit this disclosure to the Agilent Technologies Legal Department as soon as possible. No patent protection is possible until a patent application is authorized, prepared, and submitted to the Government.

## Descriptive Title of Invention:

Use of thermal inkjets for deposition of proteins and multiple reagents  
in a reaction

## Name of Project:

## Product Name or Number:

Was a description of the invention published, or are you planning to publish? If so, the date(s) and publication(s): NO

Was a product including the invention announced, offered for sale, sold, or is such activity proposed? If so, the date(s) and location(s): NO

Was the invention disclosed to anyone outside of AGILENT TECHNOLOGIES, or will such disclosure occur? If so, the date(s) and name(s): NO

If any of the above activities will occur within 3 months, call your IP attorney or the Legal Department now at 1-853-3081 or 408-853-3081.

Was the invention described in a lab book or other record? If so, please identify (lab book #, etc.):

OC notebook # 2234, pg 43-44, 50-55 PT notebook # 2735, pg 133-137

Was the invention built or tested? If so, the date:

JUL 11

Was this invention made under a government contract? If so, the agency and contract number: NO

Description of Invention: Please preserve all records of the invention and attach additional pages for the following. Each additional page should be signed and dated by the inventor(s) and witness(es).

- A. Prior solutions and their disadvantages (If available, attach copies of product literature, technical articles, patents, etc.)
- B. Problems solved by the invention
- C. Advantages of the invention over what has been done before.
- D. Description of the construction and operation of the invention (include appropriate schematic, block, & timing diagrams; drawings; samples; graphs; flowcharts; particular features; test results, etc.)

Signature of Inventor(s): Pursuant to my (our) employment agreement, I (we) submit this disclosure on this date: [ ]

Employee No.	Name	Signature	Phone	Mailstop	Entity & Lab Name
493629	Douglas D. Evers	[Signature]	857-5716	251-76	BSO
493536	Patrice Evers	[Signature]	857-5716	251-76	BSO
248495	Michael P. Curren	[Signature]	857-5729	250	BSO
493752	Douglas Evers	[Signature]	857-6211	251	BSO

(If more than four inventors, include additional information on another copy of this form and attach to this document)

<b>S1</b>		<b>INVENTION DISCLOSURE</b>		<b>COMPANY CONFIDENTIAL</b>		<b>PAGE 2 OF 2</b>		<b>RECEIVED CENTRAL FAX CENTER</b>	
Signature of Witness(es): (Please try to obtain the signature of the person(s) to whom invention was first disclosed.) The invention was first explained to, and understood by, me (us) on this date: <u>1</u>								<b>JUL 11 2007</b>	
Full Name		Signature		Date of Signature					
Lawrence W. Stanton		[Signature]							
Full Name		Signature		Date of Signature					
Bill Gate		[Signature]							
Inventor & Home Address Information: (If more than four inventors, include add. information on a copy of this form & attach to this document)									
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Deane Tisley Turner									
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6254 Main Blvd									
City									
San Jose CA 95123									
Do you have a Residential P.O. Address? P.O. BOX									
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None									
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Doug									
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## **Use of thermal inkjets for deposition of proteins and multiple reagents in a reaction**

### **Prior solutions:**

Bioanalytical microsystems, such as biosensors and assays in a microarray format or reaction chamber, often require the use of a technology that can dispense very small quantities (pico- and nanoliters) of solutions containing biomolecules. The technology must be rapid, highly reproducible, and deposit solutions with precise placement onto a given solid support. Several recent papers have described the design and construction of devices for the production of microarrays of biomolecules onto solid support (1-4).

In particular, a method is described using a conventional inkjet printer for the microdeposition of proteins (3). The black ink was removed from an HP ink cartridge and the cartridge was extensively washed with water. The cartridge was filled with the protein deposition solution using a microsyringe and sealed. The protein solution was then deposited onto a solid support and allowed to air dry, where it remained active for 1-2 weeks when stored at 4C. One problem with this method is a minimum of 2 mL of solution was needed to fill the cartridge. Unused sample could be recovered, with the exception of 200-300 uL that remained in the print head. Other problems include only a single solution can be loaded at time and loading is done manually. Similarly, Deeg et al. describe a method using bubble-jet technology for the metered application of an analytical liquid to a target. Their method is based on the manufacture of disposable jet units containing the analytical liquid in prepacked form. A preloaded jet may be cost effective, but lacks flexibility. Several different solutions can be delivered by aligning several jet elements in a row, rather than prepacking several different solutions into a single inkjet.

### **Description of the invention:**

This invention uses an inkjet for deposition of extremely small quantities of reagents directly in a reaction chamber or on a solid support. The reagents are front loaded into the inkjet head. Multiple reagents can be loaded simultaneously and the amount of material needed is 2 uL. The reagents are then spotted onto the solid support or reaction chamber. The inkjet can then be washed and reloaded with a different set of reagents.

### **Demonstration of feasibility:**

Using Biodot 3 and a Hobbes inkjet head, 2 uL of 100 ug/mL bovine albumin serum (BSA) in 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, and 0.05% SDS was loaded into 6 reservoirs of the inkjet. The concentration of the BSA solution was determined using Bradford Reagent. The head was fired multiple times, and the solution was collected for analysis (6 uL). Two microliters of the pre- and post-fired solutions were analyzed using the Caliper prototype protein LabChip assay. Figure 1 shows these results. The overlaid electropherograms show that the BSA was not degraded during the firing of the inkjet and that the protein concentration of the pre- and post-fired solutions are relatively equal. The concentration of the first load of BSA into the inkjet is slightly

lower than the pre-fired and the second loaded BSA solution, indicating that some protein does bind irreversibly in the head the first time protein is loaded.

The second experiment shows that post-fired proteins are functional. A mouse monoclonal antibody to dsDNA (500 ug/mL total protein) was loaded into 6 reservoirs of the inkjet, fired multiple times, and collected (10 uL). Two microliters were analyzed using the Caliper prototype LabChip assay. Figure 2 shows that the pre- and post-fired protein spectrums are identical. Functionality of the antibody was determined by binding to dsDNA. The pre- and post-fired antibody solutions (8 uL) were incubated in 250 uL NETG buffer (150 mM NaCl, 5 mM EDTA, 48 mM Tris-HCl, pH 7.4, 0.25% gelatin) on a cDNA microarray at room temperature for 1 hour. The slide was then washed once with 1x NETG and 3 times with 1/100x NETG. A secondary antibody conjugated to R6G (goat anti-mouse-R6G, 1/50 dilution in 250 uL NETG) was added and incubated for 1 hour at room temperature to detect binding of the primary antibody to dsDNA. The slide was washed as described above, and scanned on the Axon scanner. Figures 3 and 4 show that the pre- and post-fired antibody solutions gave similar fluorescent signals and specificity for binding the DNA. Firing the antibody through the inkjet does not appear to affect functionality.

**Experiments in progress:**

1. Washing experiments to determine if proteins can be removed from the inkjet. A solution of 5 proteins ( $M_r=116,000$ , 97,000, 66,000, 45,000, and 18,500) of known concentration will be loaded into the head, fired, and collected. The head will be washed, and loaded with buffer. The buffer will be fired and collected. The concentration of the pre- and post-fired protein and buffer solutions will be determined using the Caliper protein LabChip assay.
2. Deposition of multiple reagents on a surface for a reaction. A glass slide containing inkjet deposited cDNAs that are crosslinked to the surface will be used. Cy5-dCTP will be hand-spotted randomly onto the surface and allowed to dry. A solution containing buffer and dNTPs and second solution containing DNA polymerase will be loaded into the inkjet and fired onto the glass slide. The slide will be incubated in a humid chamber at 37C for 60 minutes to allow DNA polymerization. The slide will be washed to removed unincorporated Cy5-dCTP. The slide will then be scanned for covalently linked Cy5-dCMP to the DNA attached to the surface, indicating that the DNA polymerase synthesized DNA.

**Problems solved and advantages of the invention:**

The described invention has the following advantages:

1. Only 2 uL of each solution is needed for loading into the inkjet, significantly reducing the reagent cost.
2. Multiple different solutions can be loaded simultaneously. The number of different solutions loaded is dependent upon the type of inkjet. The multi reservoir head allows for individual components to be delivered from individual reservoirs and different reaction mixtures can be composed during the printing process.



3. The inkjet can be washed and reloaded with a different set of reagents.
4. The process can take place in a temperature and humidity controlled environment.

1. Graves, D.J., Su, H.J., McKenzie, S.E., Surrey, S., and Fortina, P. (1998) Anal. Chem. 70, 5085-5092.
2. Yershov, G., Barsky, V., Belgovskiy, A., Kirillov, E., Kreindlin, E., Ivanov, L., Parivno, S., Guschin, D. (1996) Proc. Natl. Acad. Sci. USA 93, 4913-4918.
3. Roda, A., Guardigli, M., Russo, C., Pasini, P., and Baraldini, M. (2000) Biotechniques 28, 492-496.
4. Deeg et al., (Aug. 16, 1994) US patent #5,338,688.

axonscanner\AxonData2\Peter\p

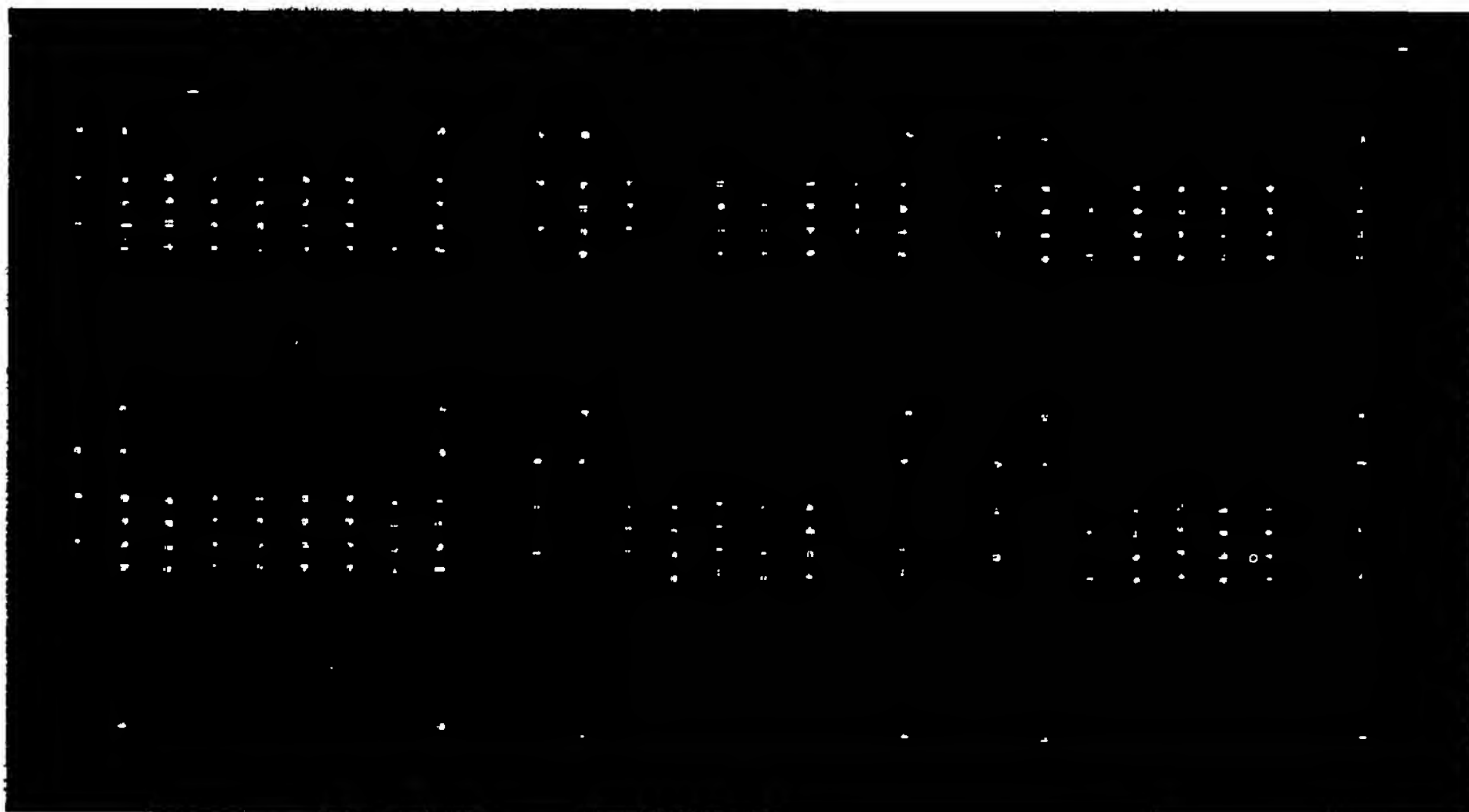
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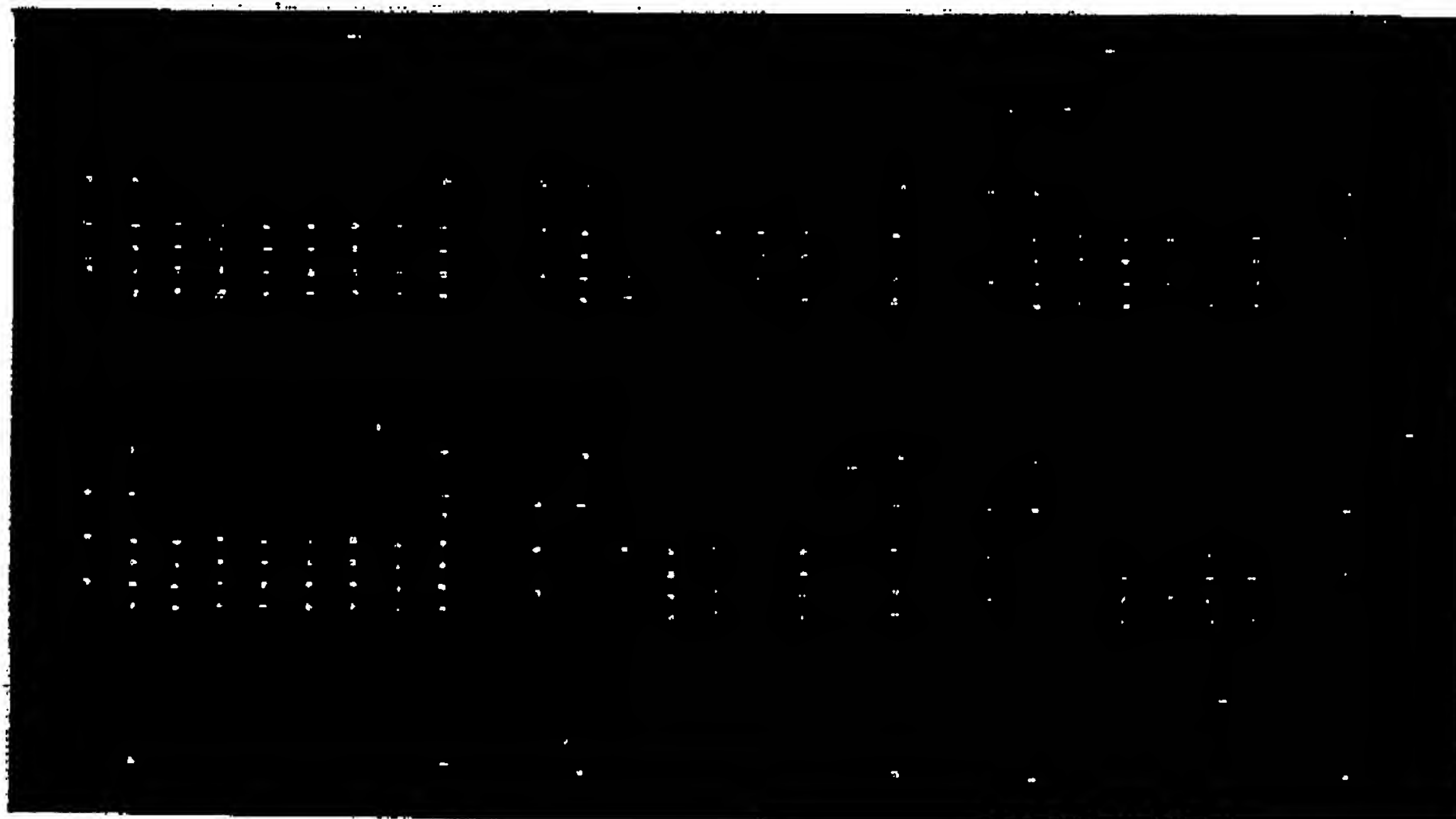
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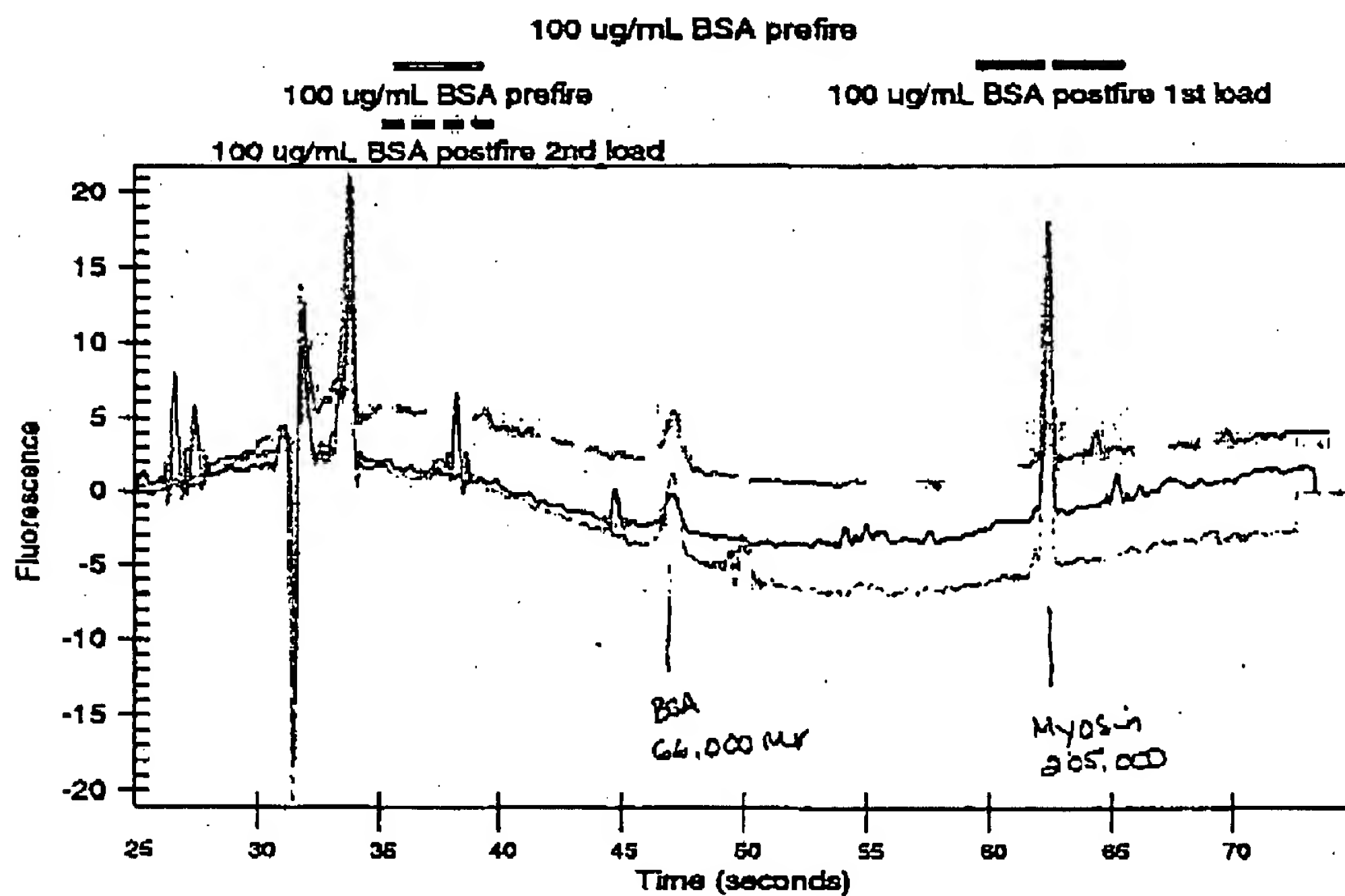


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Page 1

Data path: C:\Program Files\Agilent 2100 Bioanalyzer\Bio Sizing\Data\proteins  
Assay: Alpha Protein

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02 AM (A.01.10 S1166)



Peak	Time (sec)	Conc. Area	Size (kDa)	Conc. (ng/mL)
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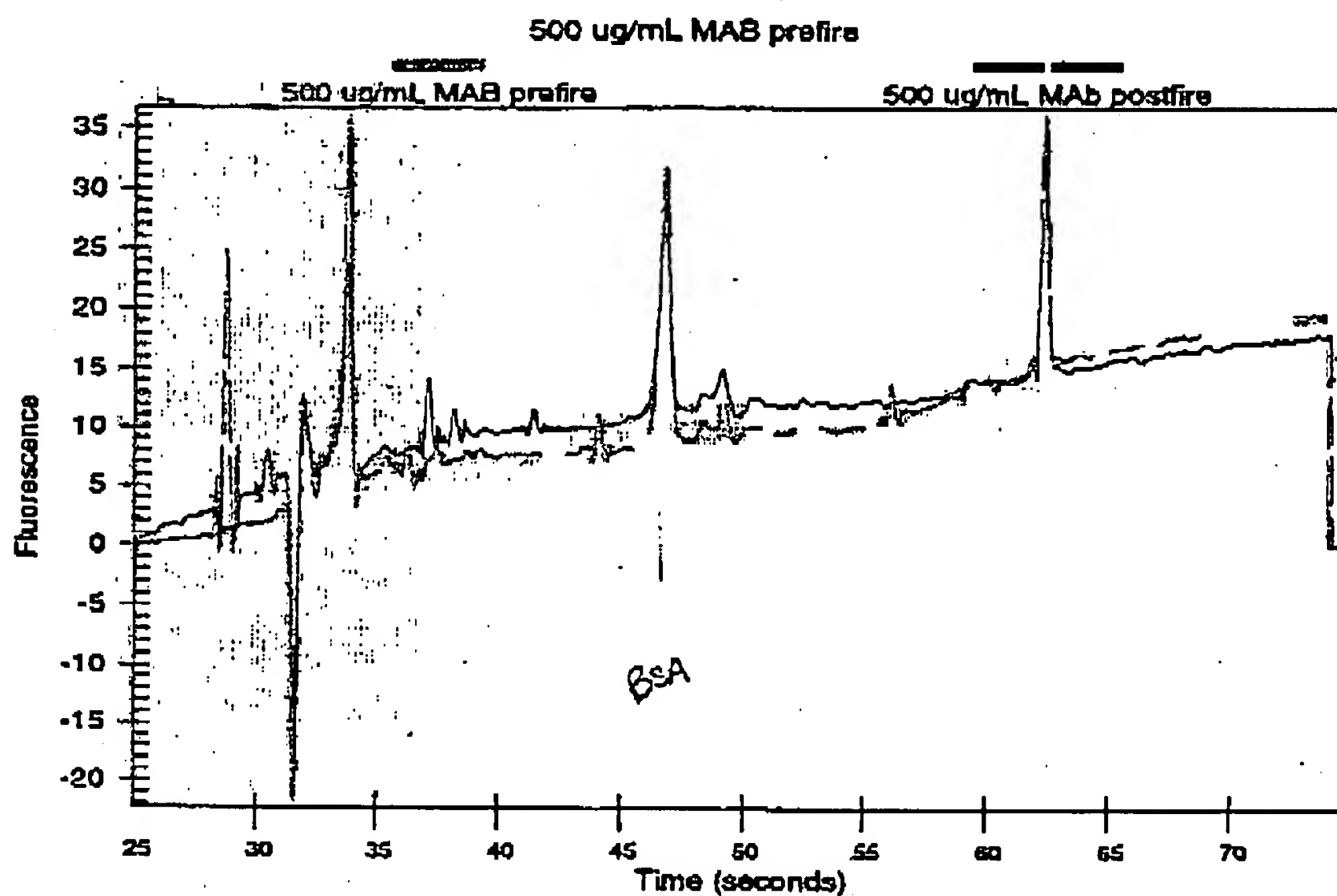
Figure 1

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Page 1

Data path: C:\Program Files\Agilent 2100 Bioanalyzer\Bio Sizing\Data\proteins  
Assay: Alpha Protein

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Peak	Time (secs)	Corr. Area	Std. Dev.	Conc. (mg/mL)
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Figure 2